Oxidative Stress and Endothelial Dysfunction in Obstructive Sleep Apnea

A Senior Honors Thesis

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by
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Background

Obstructive Sleep Apnea (OSA) is a respiratory disorder of sleep that is present in over 10% in the middle aged population [1-6]. An independent cause of hypertension[7, 8],[9], OSA accelerates the progression of existing cardiovascular disease, and increases fatal and non-fatal cardiovascular events [10-12]. OSA worsens blood pressure control[13, 14] and increases the risk of coronary disease [15] [16, 17], atherosclerosis[18-20], and stroke [21-24]. Impaired endothelial function occur in OSA before clinical manifestation of vascular disease [25]. Endothelial dysfunction, the earliest vascular abnormality predicting the development of cardiovascular disease in the general population [26-30], occurs in patients with OSA who have not yet manifested vascular disease [31-33], and in animal models prior to the appearance of OSA-induced hypertension [34]. Increased oxidant production and decreased anti-oxidant capacity occur in OSA [25]. Oxidative stress is essential for the occurrence of endothelial dysfunction in both patients and animal models[31, 35-37]. Otherwise, the mechanisms of vascular disease and endothelial dysfunction in OSA remain largely unknown.

1- Intermittent hypoxia and cardiovascular disease in OSA: A patient with OSA experiences recurrent episodes of apnea or hypopnea ranging in frequency from 5 to well over 100 events per hour. Each of these obstructive respiratory events results in an episode of hypoxia followed by re-oxygenation after the termination of the episode. In turn, each episode of hypoxia stimulates the carotid chemoreceptors[38] resulting in sympathetic nerve activation [6, 39] and a secondary surge in blood pressure[5]. The recurrence of these respiratory events and their respective recovery phases produces a characteristic pattern of nocturnal intermittent hypoxia that is unique to OSA. As a result, patients with OSA spend their sleep period in a state of intermittent hypoxia and a cycling pattern of recurrent surges of vasoconstriction. Significant experimental evidence indicates that intermittent hypoxia is a distinct pathophysiological state with a profile of biological consequences that is different from other

types of hypoxia exposure [40-43]. A memory effect (plasticity) carries over the sympathetic activation and vasoconstriction into the daytime [44] in patients with OSA. This plasticity was shown recently to be mediated by reactive oxygen species dependent pathway [40]. Thus, the nocturnal intermittent hypoxia pattern of OSA mediates the vascular response to apnea [7] [1-6, 45], and is critical for the immediate and long term cardiovascular consequences of OSA[1, 3, 7, 39]. OSA, an established cardiovascular disease, is a disorder of intermittent hypoxia, sympathetic activation, and increase vasoconstriction [46-48].

2- Endothelial dysfunction is the earliest manifestation of vascular disease in OSA: The pathogenesis of vascular disease in OSA remains insufficiently understood. OSA related abnormality in vasoregulation involves several vascular beds including cerebral perfusion [34, 49, 50], mesenteric circulation [51], and importantly limb vessels [36, 52]. In OSA, it is likely that the repetitive episodes of vasoconstriction, shear stress, and sympathetic upregulation will result in long term remodeling in the vascular walls and therefore account for such impaired vasomotion. However, impairment in vasoregulatory mechanisms occurs in the absence of such remodeling [36, 52]. In fact microcirculatory endothelial cell activation in OSA directly triggers pro-atherogenic inflammatory response[19] [53]. Thus, vascular dysregulation in OSA includes vasodilatory and vasoconstrictor responses, occurs early in the disease process, and is independent from vascular remodeling or hypertension. In particular, this vascular dysregulation of OSA appears to originate in the endothelium dependent vasomotion [33, 34, 36, 51, 52, 54]. This OSA induced impairment in vasomotion has serious implication for vascular disease and for tissue perfusion in patients with OSA.

Impaired NO mediated endothelial vasodilation is well established in OSA [33] [31]. Ip et al demonstrated impaired NO related FMD in conduit arteries (brachial) of OSA patients who were otherwise free of clinically known cardiovascular disease. This endothelial dysfunction correlated with the severity of

OSA as measured by the apnea hypopnea index (AHI), and improved after treatment of OSA [52]. A similar correlation between NO-dependent baseline vascular diameter and severity of OSA was also reported in a large population based study further supporting a cause-effect relationship [55]. Other studies evaluated resistance arterioles and found also impairment in NO related endothelial vasodilation [33]. Circulating levels of nitrite/nitrate in patients with OSA were decreased at baseline and improved with treatment with CPAP[56]. Studies demonstrated improvement in endothelial dysfunction with anti-oxidants in patients with OSA and animal models [31, 35, 36], suggesting a role for oxidative stress in the mechanism of decreased NO availability in patients with OSA. This provides parallels to other cardiovascular diseases in which oxidative stress induced endothelial dysfunction is important[57],[58]. Endothelial dysfunction and decreased NO availability is well established in OSA, and is related to increased oxidative stress.

2.a Nitric Oxide deficiency in OSA: The mechanism of NO mediated endothelial dysfunction in OSA is unknown but appears to be related to increased endothelial oxidant production [31]. Increased uptake of NO by superoxide forming peroxynitrite, an important pathway in vascular diseases, has not been directly evaluated in OSA. There are limited data on the expression or function endothelial NO synthase (eNOS), the main enzyme producing NO in the endothelium [37]. Decreased production of NO due to decreased substrate availability or dysfunction of eNOS has not yet been evaluated in OSA. Decreased availability of NO in disease states can be mediated by any one of several mechanisms, broadly categorized into decreased production, increased degradation or both [59-62]. eNOS is constitutively expressed in the endothelial cell and is responsible for most of the NO production in the endothelium. Decreased eNOS function may be due to decreased expression or inhibition of function. The decrease in NO bioavailability in OSA cannot be explained simply by decreased transcription or translation of eNOS enzyme protein. eNOS may be normal or increased, despite endothelial dysfunction, in many vascular disease states, such as diabetes[63], hypertension[64], and heart failure [65]. While, overexpression of eNOS by gene transfer can increase NO activity in the vessel wall, constitutive overexpression of eNOS accelerated atherosclerosis in association with increased oxidative stress

[66]. Overall, it is established that vascular disease occurs in the presence of higher levels of eNOS expression [66, 67].

2. b. Oxidative stress is a possible mechanism of nitric oxide deficiency in OSA: Superoxide is the most important oxidant in the endothelial cell[68-70], and can be produced by a variety of systems, including, xanthine oxidoreductase (XOR), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, Cytochrome P450 enzymes, and the arachidonic acid pathway enzymes. Of these, NADPH oxidase plays a major role in vascular endothelial cells [68, 71].The role of superoxide in the pathogenesis of vascular disease is well described [69, 70]. In the early stage of atherosclerosis, superoxide is produced from NADPH oxidase localized in the endothelium [72]. Recently, however, increasing evidence is suggesting that eNOS itself can generate superoxide under certain pathophysiological conditions. Electron transfer within the active site becomes "uncoupled" from L-arginine oxidation; instead, molecular oxygen is reduced to form superoxide [73, 74]. Superoxide generation by eNOS has been implicated in a variety of experimental and clinical vascular disease states, including diabetes [63, 75], hypertension [76], and atherosclerosis [77]. Superoxide has very high rate constant for the reaction with NO producing peroxynitrite[78]. Likewise, the newly formed peroxynitrite has a very high rate constant for the reaction with BH4, exceeding that of its reaction with most anti-oxidant systems in the endothelial cell [79, 80].

BH4 is a critical co-factor for eNOS function and structure. The importance of BH4 in the catalytic process of L-arginine oxidation and NO synthesis by eNOS is well established [81, 82]. BH4 stabilizes and donates electrons to the oxygenase domain of eNOS, as the initial step of L-arginine oxidation [83, 84]. When BH4 is limiting, electron transfer from eNOS becomes uncoupled from L-arginine oxidation, and superoxide is produced from the oxygenase domain[73]. BH4 is important for the function of eNOS, as well as for its physical stabilization [85, 86]. Decreased availability of BH4 results in dissociation between eNOS protein levels and NO production, a stoichiometric dysfunction often termed “eNOS uncoupling” [81, 82]. The most common reason for decreased BH4 availability is BH4 oxidation to BH2 in cases of abundance of peroxynitrite[87].

In patients with OSA, treatment with anti-oxidants improved endothelial dysfunction [31, 88]. Oxidative stress plays a major role in disorders of endothelial dysfunction and NO bioavailability [89-92]. Oxidative stress is critical for the pathophysiology of OSA and provides an important link between OSA and its vascular complications.

OSA is associated with increased oxidant production and decreased anti-oxidant capacity. Recent studies demonstrated increased peroxynitrite levels in OSA patients [37, 93]. The role of oxidative stress in decreased NO availability in patients with OSA is well established [31, 35]. The source and mechanism of increased oxidant production or decreased anti-oxidant capacity in OSA is unknown.

Impaired vascular regulation of the microcirculation occurs in OSA [33, 34, 36] [94]independent of hypertension; itself a disorder of the microcirculation[95, 96]. NO related endothelial dysfunction occurs in OSA as the earliest vascular abnormality prior to the manifestation of vascular disease [34, 52]and it results in impaired vasodilatory response to hypoxia[49]. We hypothesized that patients with OSA who are free of any cardiovascular disease will have early microcirculatory changes that are unique to OSA, and therefore would resolve with treatment. Given the role of oxidative stress in the vascular disease of OSA [31, 36], we were interested in evaluating the role of endothelial derived oxidants in the microcirculatory NO deficiency. Understanding these early oxidant related microcirculatory changes would give insight into the mechanism of vascular complications in OSA and its impact on perfusion of peripheral tissue. To achieve these goals, we developed several methods of evaluating the early preclinical changes in the resistance microcirculation of OSA patients.

Methods

To address our hypothesis, we performed an observational study in OSA patients and healthy controls. Patients with OSA (n=7) were recruited from the OSU sleep disorders center within 4 weeks of their diagnostic polysomnography. OSA was defined by an AHI >15 events per hours of sleep. Healthy controls (n=7) were free of sleepiness or risk factors of OSA as defined by negative Berlin questionnaire ® and negative Epworth

Sleepiness Scale (assess sleepiness). For patients with OSA and healthy controls the following exclusion criteria were met: 1)Hypertension defined by existing treatment with antihypertensives or any measurement of systolic blood pressure (SBP) above 130 mmHg, or diastolic blood pressure (DBP) above 85 mmHg; 2)Dyslipidemia defined by a value of fasting, or random cholesterol, above 180 or fasting LDL over 140 mg/dl; 3)patients with diabetes defined as existing diagnosis, hemoglobin A1C >7 or fasting glucose >110 on two separate measurements (standard fasting glucose or HbA1C criteria); 4)Coronary artery disease (CAD) defined by history of angina, coronary event or abnormal stress test; 5)Peripheral vascular disease (PVD) defined by history of stroke, claudication or abnormal ankle brachial index; and 6)Smoking. None of the female participants were concurrently pregnant or had been pregnant within the past six months.

Studies were conducted between 7-9 AM at the Cardiovascular Clinical and Translational Research Organization (CCTRO) in a quiet temperature controlled room with subjects in the fasting state ≥8 hours Doppler ultrasound studies were done first, followed by blood draw, and then the subcutaneous biopsy of the forearm was performed at the end. Only OSA patients returned after 10-12 weeks of treatment for repeat FMD, blood draw, and biopsy. Effective treatment was verified by device download.

Endothelial function evaluated by Doppler ultrasound technique and measurements of flow mediated dilation (FMD) of the brachial artery as a function of endothelial dependent dilation [89, 97]. The protocol was performed according to standard guidelines [97]. Image Acquisition was done by a linear array transducer with frequency of 7 MHz, and color spectral Doppler(GE Vivid 7). An external electrocardiogram was used. The brachial artery was imaged ≈2-12 cm above the antecubital fossa in the longitudinal plane, and a segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2D gray scale imaging. Images were captured for 2 minutes to obtain baseline reading.

Flow mediated dilation was assessed by inflating a pediatric sphygmomanometers placed on the lower forearm for 5 minutes. The longitudinal image of the artery was recorded continuously from 30 seconds before to 2 minutes after cuff deflation. FMD is considered as percent change from baseline after 30 seconds

of deflation. **Endothelial independent vasodilation** was assessed by measuring the dilation in response to a single sublingual dose of nitroglycerine (0.4 mg). All recordings were stored digitally and analyzed by two blinded observers with known inter-observer variability using specialized software (GE, Echopac Dimension). Repeated measurements were compared for analysis of FMD and NTG administration. The formulas used for calculation is as follows:

Flow mediated dilation, %= ((averaged thirty second diameter post cuff release - averaged baseline diameter)/ averaged baseline diameter) X 100. And NTG induced dilation%= ((averaged two minute post NTG administration diameter - averaged baseline diameter)/ averaged baseline diameter) X 100.

The patient's blood was then drawn by CCTRO personnel, obtaining 30 milliliters of blood.

Skin punch biopsy techniques were used to obtain a 6 mm diameter and 8mm deep cylindrical subcutaneous skin biopsy from the forearms of volunteers. Patients with OSA provided biopsies at baseline before treatment and 10-12 weeks after effective CPAP treatment, while healthy controls provided the biopsies only once. The biopsy tissue was rinsed in phosphate buffered saline (PBS) and in an RNA free solution. The tissue was then bisected into two 3mm sections. The samples were frozen in Optimal Cutting Temperature (OCT) compound at -80°C until use. One section of the tissue was used for LMPC and rtPCR study. The other 3 mm section was used for immunohistochemical analysis

Measurements:

Quantitative immunohistochemistry staining for peroxynitrite was performed on the subcutaneous biopsy. Blocks of the subcutaneous tissue were first cryosectioned into 10 µm sections using a cryostat (Leica, Bannockburn, IL) and mounted on Superfrost® plus slides (Fisher Scientific). Staining with anti nitrotyrosine polyclonal antibody (Upstate Biotechnology, Lake Placid, NY) was performed using an auto-stainer (Dako Scientific, Carpinteria, CA) in the OSU core facility. A positive slide indicating the top level of stain density possible for a vessel was created by incubating a vessel in a solution of 1mM sodium nitrite, 1 mM hydrogen

peroxide in 100 mM sodium acetate, pH 5.0 before applying the primary stain for peroxynitrite. A negative slide indicating the lower limit possible for a skin biopsy stained for peroxynitrite was created by incubating the primary antibody with 10 mM nitrotyrosine in PBS for 1 hour at room temperature and this in place of the primary antibody. Figure 1 shows an example of nitrotyrosine stain uptake in the microcirculation of an OSA patient.

The slides were scanned and the subcutaneous vessels were marked by a technician who was blinded to origin of the slides. The technician digitally dissected vessels that were less than 300 µm in diameter for all slides. Another blinded observer used image analysis software (BioImagene, Cupertino California) to quantify stain densities in the de-identified vessels. The stain density was defined as the amount of total of brown stain present in microvasculature from tissue sections compared to the blue counterstain in the tissue.

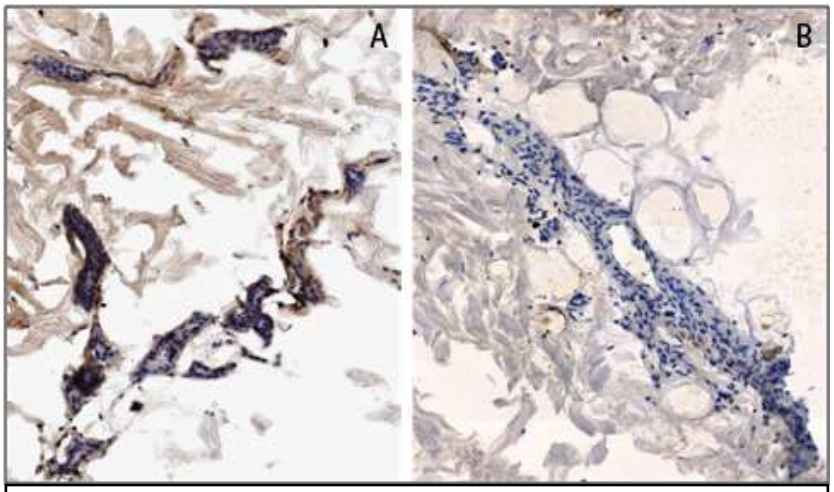

Figure 1: Peroxynitrite deposit in a microcirculatory of an OSA Patient
Brown stain indicates nitrated tyrosine residues in microcirculation.
A. A section of pre treatment tissue stained with anti-nitrotyrosine antibody;
B. A section of post treatment tissue stained with anti-nitrotyrosine antibody

The subcutaneous tissue was prepared for laser microdissection and pressure catapulting (LMPC) in order to procure HMECs according to the protocol developed by Sen et al [98] with modifications. Frozen tissue blocks were cut into 12 µm cryostat sections. Two to three sections were mounted on each RNAZap-treated thermoplastic (polyethylene napthalate)-covered glass slide (PALM Technologies, Bernreid, Germany) and treated with RNALater (Ambion, Austin, TX). The sections were stained with fluorescein-labeled UEA I (Vector Labs, Burlingame, CA). We used the laser microdissection system from PALM Technologies for LMPC. Figure 2 shows an arteriole with endothelial cells fluorescently stained that has been selected for RNA isolation via LMPC.

RNA Isolation from the LMPC samples was performed using the PicoPure RNA Isolation Kit (Arcturus, Sunnyvale, CA). RNA quantity was measured by using the NanoDrop system (NanoDrop Technologies, Wilmington, DE). The average amount of RNA per patient was 4 ng/µl.

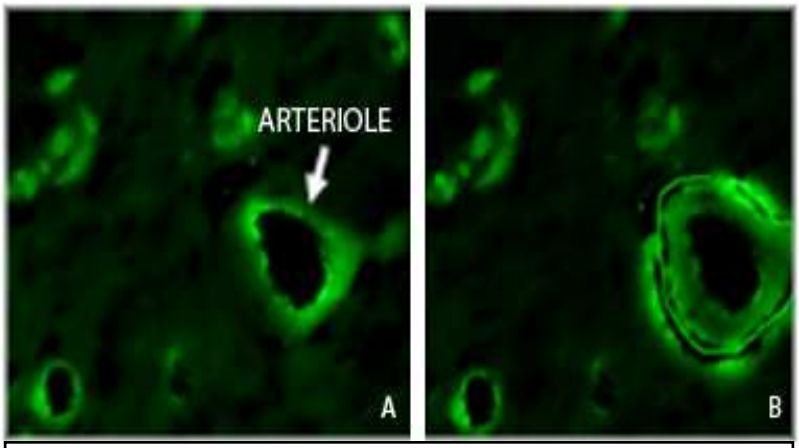

Figure 2: Microcirculatory Endothelial Cell Isolation

A. In fluorescein-labeled UEA I stained arterioles; localization of stain in microcirculatory endothelial cells is performed to enable cell selection by laser capture microdissection,

B. Area selected and marked for capture and RNA isolation for creation of cDNA library.

Levels of RNA transcription were determined by Reverse Transcription and Quantitative Real-time PCR (rtPCR): Amplified RNA was reverse transcribed into cDNA, using the SuperScript® VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). Reactions were done by using random hexamer priming. cDNA standards were used to determine relative quantities and to compare dissociation temperatures to partially ensure correct product formation. β-Actin and 18sRNA gene expression was measured to correct for differences in extraction efficiency between samples. We used the ΔCt values in analyzing the PCR results correcting to these house-keeping genes. Primers were from SuperArray Bioscience Corporation (Fredrick, MD).

Results

Characteristics of participants: Table-1 lists the baseline characteristics of patients and controls. There was no significant difference in BMI, age, or sex. For OSA patients, average CPAP use on device download was mean ± (SEM) 5.05 ± 0.83 hours. Residual AHI was 3.3±0.7 events/hour.

Table-1 Baseline characteristics of patients and controls

	Control	OSA Group Pre-treatment	Control vs. Pre Treatment
	Mean(SEM) [n]	Mean(SEM) [n]	Mean Difference(SEM) [p value]
AGE years	36.4(2.3) [7]	39.4(5.4) [7]	-3.0(5.9) [0.6]
BMI kg/m^2	30(2) [7]	35(2) [7]	-5 (3) [0.17]
GENDER (male%)	71%(18%) [7]	71%(18%) [7]	0(0.3) [1.00]
AHI	3(1) [6]	35(10) [7]	-32 (12) [0.018]

Endothelial function: FMD increased in patients after 12 weeks of CPAP treatment from 5.7 ± (0.5) to 7.3 ± (0.9) % with a difference of 1.7 ± (0.6) %, p< 0.04. Patients with OSA had lower FMD at baseline as compared to controls 5.7 ± (0.5) % versus 9.7 ± (0.6) % with a difference of 3.8 ± (0.7) %, p<0.001. After adjustment for BMI, age, and gender, the difference between OSA and control remained significant (difference=3.2 ± (1.1), p=0.02). We also explored relationships between change in FMD after treatment with BMI, AHI, and compliance, and no clear associations were found.

Peroxynitrite deposit in the microcirculation: Tissue was available for immunohistochemistry staining on 5 patients with OSA and 5 controls. Peroxynitrite stain density in the microcirculation decreased with CPAP treatment from 44.0 ± (1.6) stain density units (SDU) to 30.50 ± (2.3) SDU with a difference of-13.5 ± (2.9) SDU, p=0.009. Microcirculatory peroxynitrite stain density was increased in patients with OSA compared to controls 44.0 ± (1.6) SDU versus 21.8± (1.9) SDU with a difference of -22.3 ± (2.6) SDU, p<0.001. Figure 3 is an individual value line plot showing the peroxynitrite deposit relationship in the microvasculature between patients and controls. We also adjusted for BMI, age, sex for the comparison between patients and controls, and the adjusted difference remained significant (difference=-23.1±(6.4), p=0.02).

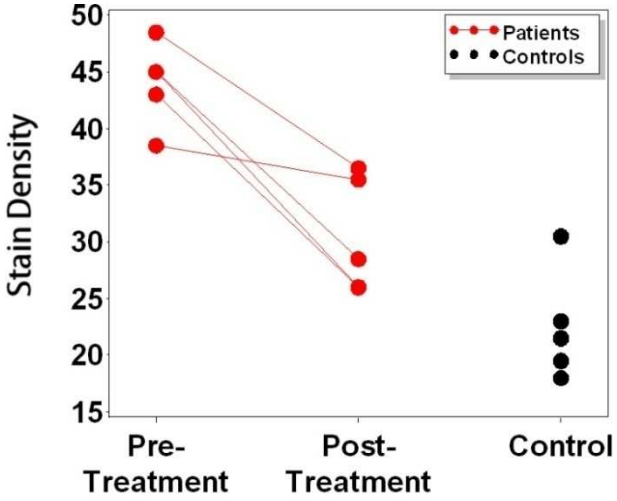

Figure 3: Peroxynitrite Deposits in the Microvascular Walls of OSA Patients and Controls

Quantitative PCR studies:

1- Transcription of Zinc, copper superoxide dismutase (SOD-1): Quantitative PCR measurement of SOD 1 revealed a decrease in expression with treatment of OSA from -4.0 to -12.1 ΔCt with a difference of -8.3 ± (2.1) ΔCt, p=0.011. Figure 4 is an

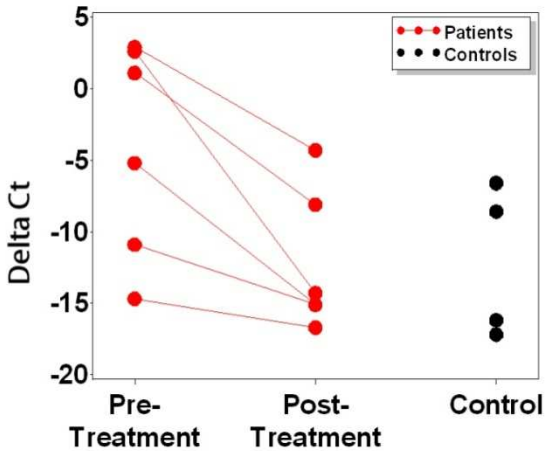

Figure 4: Superoxide Dismutase Transcription in Microvascular Endothelial Cells of Patients and Matched Controls

individual value line plot showing the relationship between transcription of SOD-1 in patients before and after treatment.

2- Transcription of endothelial nitric oxide synthase (eNOS):

There was a decrease in eNOS transcription with CPAP treatment from 5.2 to -1.3 ΔCt with a difference of -6.5 ± (2.5) ΔCt, p=0.05. Figure 5 provides the individual changes with CPAP treatment and controls.

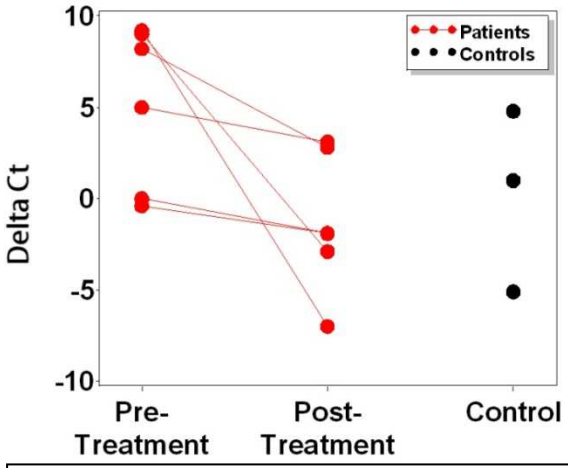

Figure 5: Endothelial Nitric Oxide Transcription in Microvascular Endothelial Cells of Patients and Matched controls

3- Transcription of inducible nitric oxide synthase (iNOS):

There was no significant difference change in iNOS RNA with CPAP treatment -4.3 ± (5.7) ΔCt at baseline to 0.6 ± (2.4) ΔCt post treatment with a difference of -4.8 ± (6.1) ΔCt, p=0.46.

Discussion

This study is the first direct evaluation of the microcirculation in OSA. We examined the subcutaneous vascular tissue from patients with OSA and low cardiovascular risk status. We found increased peroxynitrite production in the microvascular walls of OSA patients indicating overproduction of NO and superoxide in the endothelial environment. A novel endothelial cell isolation technique[98] enabled real time quantification of transcription of critical endothelial genes. The uptake of NO by superoxide explains the decreased NO availability and endothelial dysfunction in OSA. Additionally, the upregulation of SOD1 in the MVECs confirms oxidant over-production in the endothelium. The finding of upregulated eNOS at baseline in OSA patients provides a potential source for the overproduction of NO and superoxide. The findings of this study persisted after adjustment to BMI, AHI, age, sex, and hours of CPAP use.

Previous studies reported that endothelial dysfunction in OSA patients was reversible with administration of anti-oxidants[31, 35]. In this study we found direct evidence of increased peroxynitrite production in the microcirculation of OSA patients providing an explanation for the role of antioxidants. Only

one previous study reported increased circulating levels of peroxynitrite but did not localize the abnormality to the microcirculation[37]. Oxidative stress has been demonstrated in patients with OSA [25, 99]. Oxidant overproduction in OSA may be a manifestation of systemic inflammatory response and increased activated circulating leukocytes and oxidative burst[100, 101]This study supports increased oxidant production directly in the microcirculatory endothelium of OSA patients.

Peroxynitrite is the product of NO and superoxide. There are several potential sources for superoxide in the endothelium[68-70]. However, increased transcription of eNOS in the endothelial cells of OSA patients supports that eNOS may be a source of superoxide overproduction in this setting [64, 73]. Upregulation of eNOS occurs in several cardiovascular diseases in the presence of decreased NO availability [63] [64] [65]. While overexpression of eNOS by gene transfer can increase NO activity in the vessel wall, constitutive overexpression of eNOS is associated with accelerated atherosclerosis and increased oxidative stress [66]. A recent study reported decreased eNOS protein in harvested venous endothelial cells of OSA patients [37]. The technique we used provides absolute quantification of transcription of eNOS in mainly arteriolar endothelial cells. The explanation for the different findings may be related to the method and site of isolation. Our findings also support possible functional suppression of eNOS or "uncoupling" of eNOS from critical cofactors [73]. Superoxide has very high rate constant for the reaction with NO producing peroxynitrite [78]. In turn, peroxynitrite has strong predilection of oxidizing tetrahydrobiopterin [79, 80], a critical eNOS cofactor, resulting in eNOS uncoupling and production of superoxide directly. Overall, it is established that vascular disease occurs in the presence of eNOS overexpression and superoxide overproduction [64], [66], [67]. Superoxide generation by eNOS has been implicated in a variety of experimental and clinical vascular disease states, including diabetes [63, 75], hypertension [76], and atherosclerosis [77]. Studies evaluating eNOS function and superoxide production in the endothelial cells of OSA patients will be needed to confirm our findings.

The microcirculation and particularly the arterioles are critical for the peripheral vascular resistance in hypertension and vascular disease. Our functional studies were done on the brachial artery, a conduit vessel, and the structural and PCR

studies were done on subcutaneous arterioles. Correlation between endothelial dysfunction in the two beds has been confirmed [102, 103]. We did not compare the gene expression between controls and patients at baseline. We planned the gene testing to explain the treatment effect on peroxynitrite production in the microcirculation. Once we established the difference from controls for clinical variables, we only wanted to show that treatment affected mechanistic variables in the hypothesized pathway.

In this direct examination of the microcirculation, we found evidence of increased oxidant production in the microcirculation of OSA patients who are free of cardiovascular disease or risk factors. These microcirculatory changes were independent of age, weight, or sex, and were only related to OSA, since they reversed with treatment. The presence of peroxynitrite makes it highly likely that superoxide is overproduced directly in the microcirculation of OSA patients. This is further supported by the findings of upregulated SOD. Superoxide is likely to be generated directly in the endothelial cell, but this must be verified with direct measurement of superoxide production in the endothelium. This study supports that endothelial dysfunction in OSA is not related to decreased transcription of eNOS. This study also reports the first direct quantification of critical genetic markers of endothelial function in humans with OSA, providing a novel method of further studying vascular disease.

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